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Amphiphilic 4-Helix Bundles Designed for BioMolecular Materials Applications

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Artificial peptides previously designed to possess α -helical bundle motifs have been either hydrophilic (soluble in polar media) or lipophilic (soluble in non-polar media) in their overall character. The realization of these bio-inspired bundles has led to the reproduction of a variety of biomimetic functions within the appropriate media. To translate this functionality into useful biomolecular devices, the bundles must be oriented in a macroscopic ensemble, e.g., at an interface. This goal has been achieved in a new family of α -helical bundle peptides that are amphiphilic, meaning they have well-defined hydrophilic and hydrophobic domains. This peptide family binds metallo-porphyrin prosthetic groups at selected locations within these domains to impart functionality.

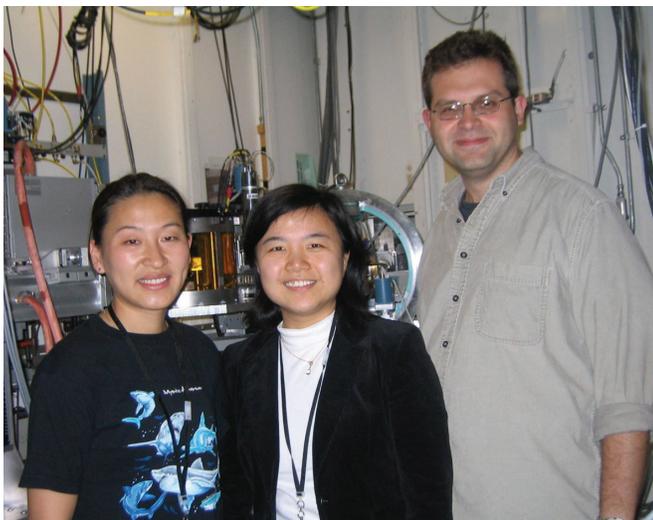
Bundles of α -helices provide a robust scaffold for binding prosthetic groups (non-amino acid portions of proteins) at selected locations within the structure, which is necessary in order to mimic the functions exhibited by biological proteins. To be used in device applications, the peptide bundles must be vectorially oriented in an ensemble, e.g., at an interface. In order to generate vectorial function across an interface, we have investigated two approaches that are designed to render the 4-helix bundles themselves amphiphilic such that they possess both a hydrophilic and a hydrophobic domain along the length of each bundle.

In the first approach, each α -helix is designed to be amphipathic, i.e. having polar and non-polar faces. However, the amino acid sequence was oriented such that when the non-polar faces of each amphipathic helix were apposed in the 4-helix bundle over the first m-residues of each helix, the polar faces were apposed over the last n-residues. Thus,

a 4-helix bundle with parallel (or *syn*) topology would possess a charged-polar exterior with a non-polar interior over the first m-residues, thereby forming the hydrophilic domain of the bundle, and a non-polar exterior with an uncharged-polar interior over the last n-residues, thereby forming the hydrophobic domain. The first of these amphiphilic 4-helix bundles was designated Amphiphilic Protein zero (AP0). AP0 can possess *bis*-histidyl metalloporphyrin binding sites at two positions of the sequence, which are separated by

fourteen residues within the larger hydrophilic portion of the 4-helix bundle.

Alternatively, these amphiphilic peptides (the second case) can possess a hydrophobic domain that utilizes a sequence of exclusively non-polar amino acids. Like AP0, these amphiphilic peptides can possess *bis*-histidyl metalloporphyrin binding sites within the hydrophilic portion of the 4-helix bundle. Importantly, they can also possess *bis*-histidyl metalloporphyrin binding sites in the hydrophobic portion of the bundle. These two new types of amphiphilic 4-helix bundles now allow for the positioning of prosthetic groups on either side or both sides of the interface. This is essential for the vectorial electron transfer across the interface, which creates an electrochemical potential. This transforms a designed molecular property at the microscopic level into a material property of the interface at the macroscopic level.



Authors (from left): Ting Xu, Shixin Ye, and Joseph Strzalka

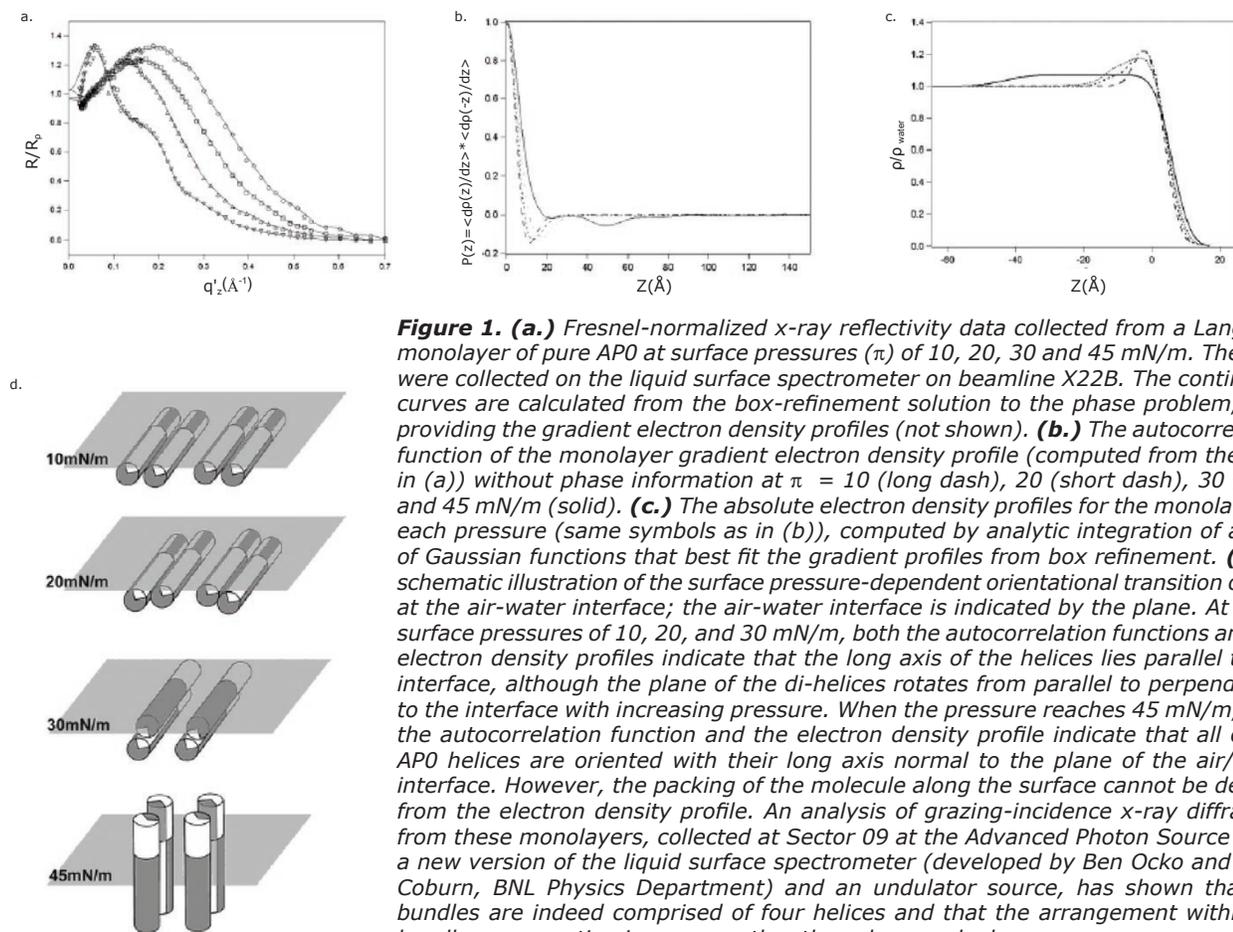


Figure 1. (a.) Fresnel-normalized x-ray reflectivity data collected from a Langmuir monolayer of pure AP0 at surface pressures (π) of 10, 20, 30 and 45 mN/m. The data were collected on the liquid surface spectrometer on beamline X22B. The continuous curves are calculated from the box-refinement solution to the phase problem, thus providing the gradient electron density profiles (not shown). (b.) The autocorrelation function of the monolayer gradient electron density profile (computed from the data in (a)) without phase information at $\pi = 10$ (long dash), 20 (short dash), 30 (dot), and 45 mN/m (solid). (c.) The absolute electron density profiles for the monolayer at each pressure (same symbols as in (b)), computed by analytic integration of a sum of Gaussian functions that best fit the gradient profiles from box refinement. (d.) A schematic illustration of the surface pressure-dependent orientational transition of AP0 at the air-water interface; the air-water interface is indicated by the plane. At lower surface pressures of 10, 20, and 30 mN/m, both the autocorrelation functions and the electron density profiles indicate that the long axis of the helices lies parallel to the interface, although the plane of the di-helices rotates from parallel to perpendicular to the interface with increasing pressure. When the pressure reaches 45 mN/m, both the autocorrelation function and the electron density profile indicate that all of the AP0 helices are oriented with their long axis normal to the plane of the air/water interface. However, the packing of the molecule along the surface cannot be derived from the electron density profile. An analysis of grazing-incidence x-ray diffraction from these monolayers, collected at Sector 09 at the Advanced Photon Source using a new version of the liquid surface spectrometer (developed by Ben Ocko and Scott Coburn, BNL Physics Department) and an undulator source, has shown that the bundles are indeed comprised of four helices and that the arrangement within the bundle cross-section is square rather than close-packed.

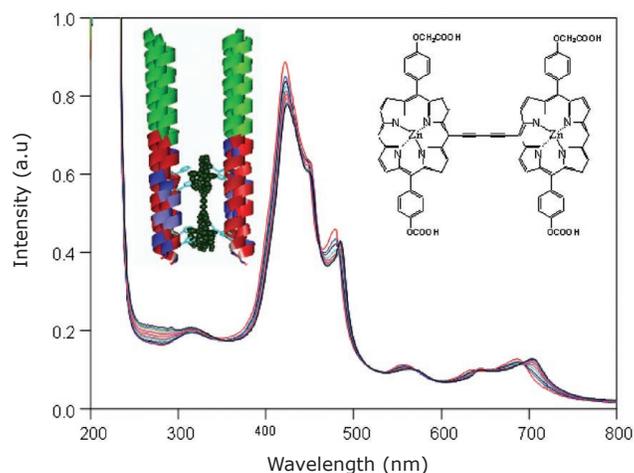


Figure 2. The amphiphilic 4-helix bundles can bind not only metallo-porphyrin prosthetic groups, but also non-biological prosthetic groups possessing much more extended π -electron systems. These non-biological prosthetic groups have the advantage of including the electron donor and the electron acceptor within the same prosthetic group, which is bound to the 4-helix bundle via only a single bis-histidyl site. An example of such a prosthetic group (synthesized and characterized by Prof. Michael Therien's research group in the Chemistry Department at the University of Pennsylvania) is shown, symmetric in this instance, with its anticipated location within AP0; the hydrophobic domain of the bundle shown in green. The changes (red-shifts) in the rich electronic spectrum of this prosthetic group upon binding to the peptide are also shown. In addition, the vectorial incorporation of this symmetric prosthetic group into the amphiphilic 4-helix bundle via a single bis-histidyl site breaks the symmetry, which should result in an exceptionally large molecular hyperpolarizability, as exhibited by closely related asymmetric, extended π -electron systems. The vectorial orientation of the 4-helix bundles in a two-dimensional ensemble at an interface between polar and non-polar media should then allow large second-order non-linear optical phenomena to be generated at the interface.